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Poly(hydroxyalkanoate) Biosynthesis from Crude Alaskan Pollock (*Theragra chalcogramma*) Oil

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Abstract Six strains of *Pseudomonas* were tested for their abilities to synthesize poly(hydroxyalkanoate) (PHA) polymers from crude Pollock oil, a large volume byproduct of the Alaskan fishing industry. All six strains were found to produce PHA polymers from hydrolyzed Pollock oil with productivities (P; the percent of the cell mass that is polymer) ranging from 6 to 53% of the cell dry weight (CDW). Two strains, *P. oleovorans* NRRL B-778 (P = 27%) and *P. oleovorans* NRRL B-14682 (P = 6%), synthesized poly(3-hydroxybutyrate) (PHB) with number average molecular weights (M_n) of 206,000 g/mol and 195,000 g/mol, respectively. Four strains, *P. oleovorans* NRRL B-14683 (P = 52%), *P. resinovorans* NRRL B-2649 (P = 53%), *P. corrugata* 388 (P = 43%), and *P. putida* KT2442 (P = 39%), synthesized medium-chain-length PHA (*mcl*-PHA) polymers with M_n values ranging from 84,000 g/mol to 153,000 g/mol. All *mcl*-PHA polymers were primarily composed of 3-hydroxyoctanoic acid ($C_{8:0}$) and 3-hydroxydecanoic acid ($C_{10:0}$) amounting to at least 75% of the total monomers present. Unsaturated monomers were also present in the *mcl*-PHA polymers at concentrations between 13% and 16%, providing loci for polymer derivatization and/or crosslinking.

Keywords Biopolymer · Medium-chain length PHA · Pollock oil · Poly(3-hydroxybutyrate) · *Pseudomonas*

Introduction

The Alaskan fishing industry is responsible for approximately two-thirds of the total wild fish consumed by humans in the United States. However, only a fraction of each fish (about one-third) is actually eaten while the rest (including the viscera, heads, skin etc.) remains as a byproduct of the industry. In the years between 2000 and 2004, the average total harvest of Walleye Pollock (*Theragra chalcogramma*), Salmon (Pink; *Oncorhynchus gorbuscha,* Chinook; *O. tshawytscha*, Chum; *O. keta*, Coho; *O. kisutch*, Sockeye; *O. nerka*) and Pacific Cod (*Gadus macrocephalus*) (major species harvested from Alaskan waters) was estimated at 2 million metric tons per annum which resulted in more than 1 million metric tons of byproducts from these species alone [1]. Some processing plants do convert these byproducts into usable materials. Specifically, those byproducts from cold-water marine fish with high-fat livers can be processed into fish oil, a good source of omega-3 fatty acids, and fish protein which can be used as feed ingredients for aquaculture, livestock and in some cases human consumption. The dilemma is that these products have limited profit margins. This problem is exacerbated for smaller or at-sea processors where, because of the economics, more than half of all fish processing byproducts go unutilized and are actually returned to the ocean to be consumed by other marine creatures. In an attempt to increase the value of these fish byproducts, new uses are currently being sought. One potential outlet that is currently showing promise is the gelatin recovered from fish skins can be processed into thin films with lower

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gelling temperatures than gelatins derived from mammalian sources, thus making them potentially more suitable for low temperature applications [2]. In fact, the film properties of these fish-derived gelatins provide better barrier properties towards water vapor and offer better protection against oxidation [3].

In an attempt to further add value to these byproducts our lab has been involved in their utilization as carbon feedstocks for the bacterial production of poly(hydroxyalkanoates) (PHAs). PHAs are bacterial polyesters that are synthesized and stored as intracellular granules by many microorganisms as carbon and energy storage materials. Presently, there are upwards of 150 different structural analogs of PHA polymers, all dependent on the specific organism used to produce them and the carbon substrate on which the organisms are grown [4]. Pseudomonads, in general, are considered to be a metabolically diverse class of microorganisms. In fact, PHAs have been produced from a variety of *Pseudomonas* strains using simple sugars [5, 6], free fatty acids [7–10], and triacylglycerols [11–13]. The cost of the carbon substrate reportedly contributes to >50% of the production cost of most bioproducts [14]; therefore, the use of inexpensive renewable agricultural and industrial coproducts as feedstocks could be a tremendous advantage to the economics of PHA polymer production provided productivities remain elevated. Recently, we demonstrated that crude glycerol (derived from the biodiesel industry) [15] and soy molasses (a major coproduct from soybean processing) [16] can be utilized as substrates for the production of PHA polymers. Since these coproducts are available in abundance and are comparatively inexpensive relative to other potential substrates, the use of these materials may lead to a diminished overhead for PHA production.

PHAs are grouped into two major categories: short-chain-length PHA (*scl*-PHA), where the repeat units of the polymer are β-hydroxy fatty acids of 4–5 carbon units; and medium-chain-length PHA (*mcl*-PHA), where the repeat units are $\geq$ 6 carbons in length. The *scl*-PHAs are thermoplastic-like because of their comparatively high crystallinity and exhibit properties comparable to some of the petrochemical-based polymers such as polypropylene (PP) and polyethylene (PE). On the other hand, *mcl*-PHAs have minimal crystallinity and hence are amorphous and exhibit elastomeric and/or free-flowing properties. Because PHAs are environmentally benign and can be produced by fermentation of renewable feedstocks, they are considered as attractive "green" substitutes for petroleum-derived polymers in many applications such as medicine, drug-delivery agents, agriculture and horticulture, fibers, and other consumer products [17, 18]. In this paper, we describe the use of crude Pollock oil as a new potential low-cost feedstock for PHA production in our attempt to help reduce the cost of the fermentative production of PHA polymers and concurrently add value to Alaskan fish byproducts.

Materials and Methods

Materials

All simple salts were obtained from Sigma-Aldrich Chemical Company (St. Louis, MO). The silylation reagent (N,O-bis(trimethylsilyl)-trifluoroacetimide; BSTFA) and pyridine (silylation grade) were purchased from Pierce Chemical Company (Rockford, IL). All solvents were HPLC grade and purchased from J. T. Baker (Phillipsburg, NJ). *Pseudomonas* strains were obtained as follows: *P. resinovorans* NRRL B-2649 (Pr-2649) and *P. oleovorans* strains NRRL B-778 (Po-778), NRRL B-14682 (Po-14682), and NRRL B-14683 (Po-14683) were obtained from the ARS Culture Collection (National Center for Agricultural Utilization Research; NCAUR, Peoria, IL). *Pseudomonas corrugata* 388 (Pc-388), originally isolated from alfalfa roots by F.L. Lukezic (Pennsylvania State University, University Park, PA), was obtained from Dr. W. F. Fett (Eastern Regional Research Center/ARS/USDA, Wyndmoor, PA). *P. putida* KT2442 (Pp-2442) was a gift from Professor R. A. Gross (Polytechnic University, Brooklyn, NY). The crude Pollock oil was supplied by Dr. P. J. Bechtel (USDA/ARS Subarctic Research Unit, Fairbanks, AK).

Pollock Oil Saponification

The Pollock oil fatty acids were obtained by chemical hydrolysis. The hydrolysis was performed on 350 g of crude Pollock oil to which was added 720 mL of solution containing 120 g NaOH, 400 mL deionized water, 400 mL ethanol, and 2.5 g ethylenediaminetetraacetic acid (EDTA). This solution was allowed to stir vigorously at 45 °C for 30 min after which 150 mL of deionized water and 1 L of hexane were added and the solution acidified with sulfuric acid to pH 1.5. The aqueous phase was washed again with 1 L of hexane and discarded. The hexane washes, containing the free fatty acids, were combined and washed twice with 500 mL deionized water to remove any residual sulfuric acid and the hexane fraction rotary-evaporated until almost dry. The resulting mixture was transferred to a pre-weighed jar and dried under vacuum to constant weight.

Fermentation Conditions

Each of the above-mentioned bacterial strains (6) was tested for its ability to synthesize PHA polymers from hydrolyzed crude Pollock oil. The inocula for each experiment was prepared by asceptically adding 1 mL of

each bacterial strain from frozen cryovials into separate 125 mL Erlenmeyer flasks containing 50 mL of sterile Luria-Bertani (LB) broth and incubating each flask at 30 °C with rotary shaking at 250 rpm for 24 h. Five milliliter aliquots of each bacterial strain from the overnight LB cultures were then asceptically transferred into separate sterile 500 mL vessels in a Sixfors fermentation system (Appropriate Technical Resources Inc., Laurel, MD) containing 500 mL volumes of medium E* (pH 7.0) (for medium composition see reference 7) and 2% Pollock oil free fatty acids as the sole carbon source (Pollock oil free fatty acids were filter sterilized through a 0.45 μm filter prior to addition into the fermentation vessels). Incubation of each vessel was carried out at 30 °C with an impeller speed of 400 rpm and aeration at 1 standard liter per minute (SLPM) for 3 days. At the appropriate time, cells from each fermentation vessel were collected by centrifugation (8,000*g*, 4 °C, 10 min) and washed once with cold deionized water. Cells were then lyophilized to dryness and weighed to obtain the dry cell weight (CDW).

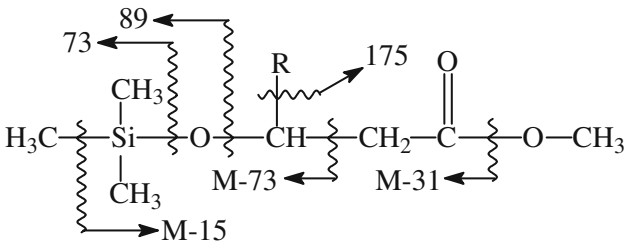
Polymer Isolation

The dry cells from each bacterial strain were extracted in 100 mL of chloroform to separate the cellular material from the PHA polymers. This extraction was conducted at 30 °C overnight with shaking at 250 rpm. The cellular debris was removed by vacuum filtration through Whatman #1 filter paper and the chloroform fraction rotary-evaporated to dryness to give the crude polymer. The crude polymer was redissolved in a small volume of chloroform and reprecipitated by dropwise addition into cold methanol. The polymer was recovered, placed into a tared vial and dried in vacuo for 24 h.

Instrumental Procedures

Fatty acid content of the hydrolyzed crude Pollock oil was determined by gas chromatography (GC) of the fatty acid methyl ester (FAME) derivatives. Fatty acids were identified by retention time-comparison to known FAME standard mixtures. The FAMEs were prepared for analysis by dissolving 100 mg of crude Pollock oil in 5 mL of methyl *t*-butyl ether. Five milliliter of a 2% sulfuric acid in methanol solution was added to the lipid solution and the mixture heated at 70 °C for 1 h in a closed vessel. After cooling, 5 mL of a 5% aqueous NaCl solution was added to the reaction mixture and the top layer removed and placed into a separate vial. The aqueous layer was extracted twice with 5 mL of hexane and the organic extracts were combined, washed with 5 mL of an aqueous 5% $NaHCO_3$ solution, and placed into a separate vial. These extracts were then dried with anhydrous Na_2SO_4. After drying, the samples were filtered through a 0.45 μm filter, evaporated to oil under nitrogen and 4 μL of each sample was diluted with 1 mL of hexane for subsequent analysis using an HP5890 GC with a 7673 autoinjector, an SP 2340 column (30 m × 0.25 μm *i.d.*, 0.2 μm film), flame ionization detection (250 °C), split injection (220 °C, ratio of 5:1) and a temperature program of 170 °C for 10 min, after which the temperature was increased at 2 °C/min up to 200 °C and held for 10 min (total run time 35 min). Helium was used as the carrier gas at constant flow.

PHA repeat unit compositions were determined by gas chromatography/mass spectrometry (GC/MS) of the silylated 3-hydroxymethyl esters obtained by acid-catalyzed methanolysis [7]. The liberated 3-hydroxymethyl esters were silylated by reacting 10 μL of each sample with 250 μL BSTFA and 200 μL pyridine. The mixtures were heated at 70 °C for 30 min and allowed to cool to room temperature. Finally, 150 μL of hexane was added to each sample and the samples analyzed by GC/MS as described elsewhere [19]. Electron impact fragmentation of silylated 3-hydroxymethyl esters occurred as shown below:



Percent composition was obtained by selecting the 175 ion (indicative of silylated 3-hydroxymethyl esters) and identifying the ions corresponding to the molecular ion (M) – 15 amu ($–CH_3$), M – 31 amu ($–OCH_3$) and M – 73 amu ($–CH_2COOCH_3$).

Molecular weight analyses were performed using gel permeation chromatography (GPC) on a Waters HPLC system equipped with a Waters Model 717 Plus autosampler, a Waters Model 1525 binary HPLC pump, a column heater module, and a Waters 2414 refractive index detector (RID). Chloroform was used as the mobile phase and temperature throughout the system was maintained at 35 °C. Two Styragel columns, HR-4E and HR-1 (7.8 × 300 mm, 5 μm particle size), were connected in series. All PHA polymers were dissolved at a concentration of precisely 5 mg/mL, and the flow rate was 1 mL/min. Injection volumes for all standards and samples were 50 μL. Molecular weight determinations were made using polystyrene as the calibration standard.

Results and Discussion

Six strains of *Pseudomonas* were screened for their ability to utilize crude Pollock oil as a substrate for the synthesis of PHA polymers. Each bacterial strain was selected due to its previously known capacity to synthesize PHA from various unrelated carbon sources. Prior work on these bacterial strains had shown that two of the organisms (Po-778 and Po-14682) possess the genetic framework to synthesize *scl*-PHA polymers (specifically poly-3-hydroxybutyrate; PHB) from such substrates as simple sugars, free fatty acids [20] and glycerol, the latter either in its pure form or as the major component of the biodiesel coproduct stream [15, 21]. The other four strains (including Po-14683, Pc-388, Pr-2649 and Pp-2442) are each reportedly capable of synthesizing *mcl*-PHA from many of the same substrates [16, 22, 23]. In addition, wildtype Pr-2649 and a genetically altered form of Pc-388 have also been reported to synthesize *mcl*-PHA polymers from intact triacylglycerols [12, 13]. In this study, the capability of each bacterial strain to grow and generate PHA polymers from hydrolyzed crude Pollock oil was investigated in an effort to produce assorted PHA polymers from a single carbon source and at the same time help to impart additional worth to a coproduct stream that is currently undervalued. Accordingly, each of the bacterial strains was incubated in the presence of 2% saponified crude Pollock oil in a well defined media (media E*) that had been formulated for improved PHA synthesis in *Pseudomonas*.

The fatty acid content of marine oils, particularly from fish, is based on the habitat and diet of the individual fish species. The fatty acid content (those fatty acids present in concentrations $\geq$1%) of the crude Pollock oil used in this study can be seen in Table 1. Gas chromatography showed that the fatty acid chain lengths ranged from 14 to 22 carbon units and that the predominant fatty acid species present in the oil consisted of monounsaturated octadecanoic acid (C18:1; 28%) that was actually present in the form of 2 isomers, oleic acid (C18:1$^{\Delta 9}$, 21%) and vaccenic acid C18:1$^{\Delta 11}$, 7%) (Fig. 1). A relatively large percentage (32%) of the fatty acids were $\geq$20 carbons in length and of those, 94% were at least monounsaturated. Both the omega-3 (α-linolenic acid, ALA; C18:3$^{\Delta 9,12,15}$, eicosatetraenoic acid, ETA; C20:4$^{\Delta 8,11,14,17}$, eicosapentaenoic acid, EPA; C20:5$^{\Delta 5,8,11,14,17}$, and docosahexaenoic acid, DHA; C22:6$^{\Delta 4,7,10,13,16,19}$) and omega-6 (arachidonic acid; C20:4$^{\Delta 5,8,11,14}$) fatty acids were represented at concentrations of 35 and 1%, respectively.

All of the bacterial strains grew and produced PHA polymers from hydrolyzed crude Pollock oil. The CDWs ranged from 1.7 to 4.7 g/L and PHA yields from 0.1 to 2.5 g/L depending on the bacterial strain and type of polymer produced (Table 2). The absolute content of the PHA polymers was determined by GC/MS of the silylated 3-hydroxymethyl esters obtained after acidic methanolysis. Figure 2 shows representative examples of the GC/MS data derived from 2 different PHA polymers. The chromatograms depicted are of the PHA polymers produced by

Table 1 The major fatty acids of crude Pollock oil prior to inoculation and after the bacterial growth of 6 *Pseudomonas* strains

Fatty acid type	Fatty acid content (mol%)[a]						
	Pollock oil	Po-14682[b]	Po-778[b]	Po-14683[b]	Pp-2442[b]	Pc-388[b]	Pr-2649[b]
Myristic acid	5	2	4	1	1	1	–
Palmitic acid	14	14	14	10	6	3	6
Palmitoleic acid	6	5	7	1	1	–	1
Heptadecanoic acid	2	3	3	4	4	5	4
Stearic acid	3	4	4	5	4	4	4
Oleic acid	21	23	22	23	17	13	20
Vaccenic acid	7	10	10	8	8	5	8
α-Linolenic acid (ALA)	10	11	10	13	14	15	14
Eicosenoic acid	3	5	5	6	6	7	6
Eicosatetraenoic acid (ETA)	9	10	11	17	17	20	15
Arachidonic acid (AA)	1	2	1	1	1	2	2
Eicosapentaenoic acid (EPA)	10	5	5	4	10	14	11
Heneicosanoic acid	2	2	–	–	1	–	–
Erucic acid	1	2	1	1	1	2	2
Docosahexaenoic acid (DHA)	6	2	2	7	9	9	7

[a] Fatty acid content refers to the relative concentration of each fatty acid before and after bacterial growth

[b] Bacterial strain designations are *P. oleovorans* NRRL B-14682 (Po-14682), *P. oleovorans* NRRL B-778 (Po-778), *P. oleovorans* NRRL B-14683 (Po-14683), *P. putida* KT2442 (Pp-2442), *P. corrugata* 388 (Pc-388) and *P. resinovorans* NRRL B-2649 (Pr-2649)

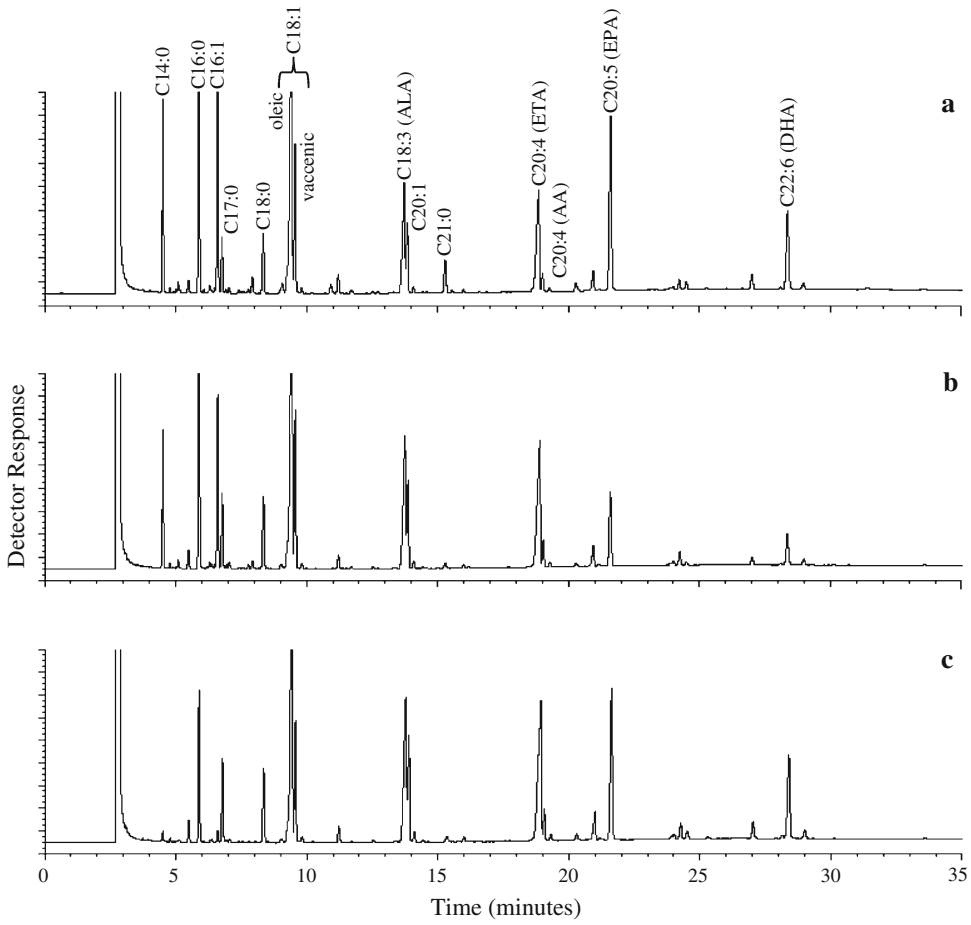


Fig. 1 Gas chromatograms of the hydrolyzed crude Pollock oil prior to inoculation (**a**), and after growth of Po-778 (**b**) and Pr-2649 (**c**). *Note*: the detector response scale is identical in all 3 GC chromatograms

Table 2 Cell growth and PHA polymer production characteristics of 6 *Pseudomonas* strains grown on hydrolyzed crude Pollock oil

Bacterial strain[a]	CDW[b] (g/L)	PHA Yield (g/L)	Pollock oil (% used)
Po-14682	1.7	0.1	46
Po-778	2.6	0.7	42
Po-14683	4.6	2.4	64
Pp-2442	3.8	1.5	61
Pc-388	4.4	1.9	69
Pr-2649	4.7	2.5	69

[a] For definitions of bacterial strain abbreviations see footnote from Table 1

[b] CDW = cell dry weight

Po-778 (Fig. 2a) and Pr-2649 (Fig. 2b). It was evident that the polymer produced by Po-778 consisted of a single monomer-type as evidenced by a single chromatographic peak, while the polymer produced by Pr-2649 was more complex and, in fact, was composed of 9 structurally distinct monomers. By examining the mass spectral data from the peaks present in each chromatogram (as described in the Materials and Methods section) the absolute chemical makeup of each PHA polymer was determined. The mass spectral data of the individual peaks present in the GC of the PHA polymers derived from Po-778 and Po-14682 showed M-15, M-31, and M-73 ions of 175 amu, 159 amu and 117 amu, which correspond to silylated 3-hydroxybutyrate methyl ester (Fig. 2a). These results indicated that the PHA polymers produced by Po-778 and Po-14682 were, in fact, PHB. Conversely, the GC/MS data from the PHA polymers produced by Pr-2649, Po-14683, Pc-388 and Pp-2442 each contained 9 distinct peaks eluting between 5.8 and 14.5 min (Fig. 2b). Analysis of the mass spectra from each of these peaks revealed the presence of 9 different monomers ranging in length from C6 to C14 that comprised each PHA polymer. This result indicated that each of these polymers was an *mcl*-PHA and was composed primarily of 3-hydroxyoctanoic acid ($C_{8:0}$) and 3-hydroxydecanoic acid ($C_{10:0}$) monomers but with significant monomer chain lengths up to 14 carbon units and between 13 and 16% of the total monomers containing some degree of unsaturation (Table 3). This unsaturation can be beneficial to *mcl*-PHA polymer processing as these olefinic sites provide loci for further chemical (epoxidation, per-oxidation) and/or physical (crosslinking) modification, valuable options when tuning the properties of these materials. The monomer with the highest degree of

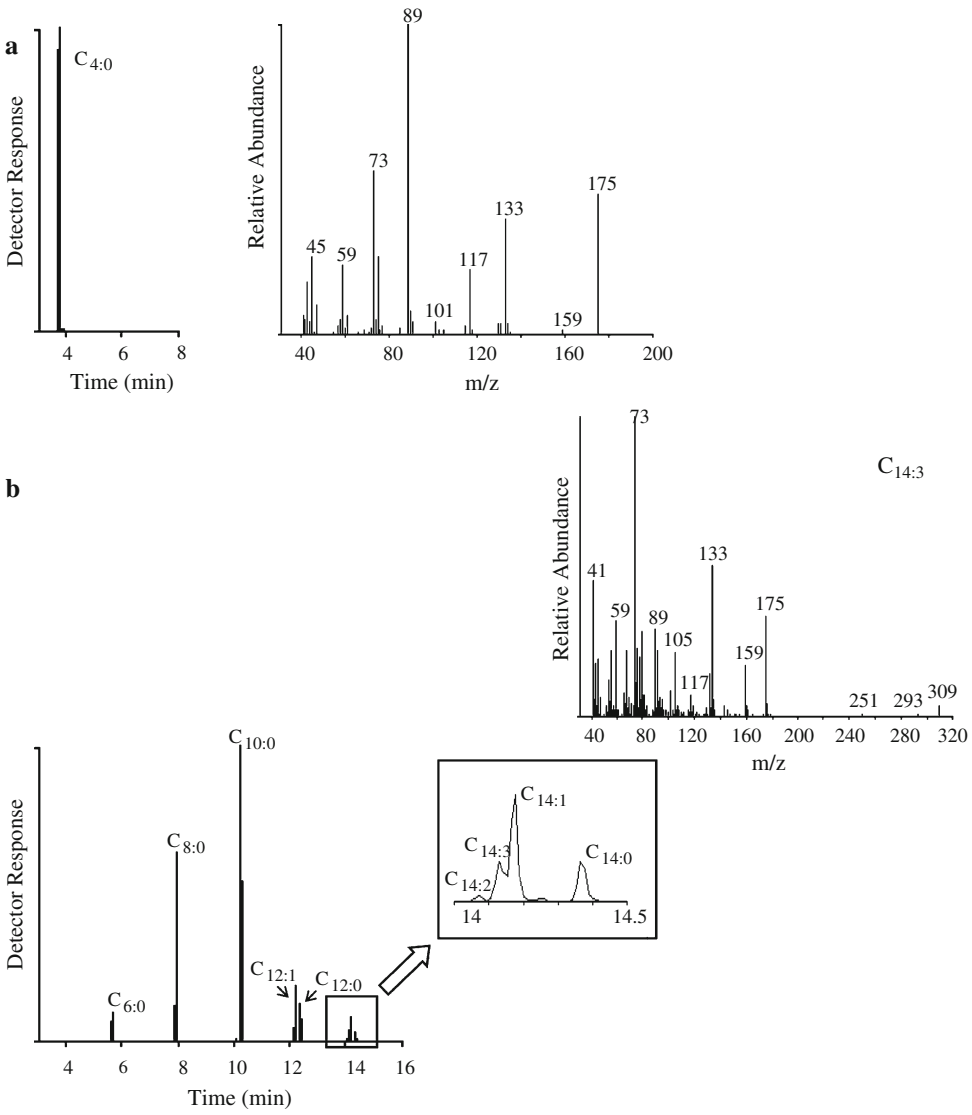


Fig. 2 Gas chromatograms and select mass spectra of the PHA polymers synthesized by Po-778 (**a**) and Pr-2649 (**b**). *Note*: The GC analysis of the polymer synthesized by Po-778 showed a single peak, indicative of a homopolymer whose mass spectra revealed 3-hydroxybutyric acid as the polymer component. Analysis of the polymer from Pr-2649 revealed a mixed copolymer with monomer chain lengths up to 14 carbon units and some unsaturation. The mass spectrum shown is of the peak that eluted at approximately 14.13 min and was determined to be 3-hydroxytetradecatrienoic acid ($C_{14:3}$)

Table 3 β-hydroxyalkanoate repeat unit composition of PHA polymers synthesized from hydrolyzed crude Pollock oil by 6 *Pseudomonas* strains

Bacterial strain[a]	β-hydroxymethyl esters[b] (mol%)									
	$C_{4:0}$	$C_{6:0}$	$C_{8:0}$	$C_{10:0}$	$C_{12:0}$	$C_{12:1}$	$C_{14:0}$	$C_{14:1}$	$C_{14:2}$	$C_{14:3}$
Po-14682	100	–	–	–	–	–	–	–	–	–
Po-778	100	–	–	–	–	–	–	–	–	–
Po-14683	–	2	55	22	4	8	1	4	Tr	4
Pp-2442	–	3	60	21	3	6	1	4	Tr	3
Pc-388	–	3	55	23	4	7	1	4	Tr	3
Pr-2649	–	3	27	48	7	8	1	5	Tr	1

[a] For definitions of bacterial strain abbreviations see footnote from Table 1

[b] Values typically have standard errors of <5%. Tr, trace amounts detected (≤0.5 mol%). 3-hydroxybutyrate ($C_{4:0}$), 3-hydroxyhexanoate ($C_{6:0}$), 3-hydroxyoctanoate ($C_{8:0}$), 3-hydroxydecanoate ($C_{10:0}$), 3-hydroxydodecanoate ($C_{12:0}$), 3-hydroxydodecenoate ($C_{12:1}$), 3-hydroxytetradecanoate ($C_{14:0}$), 3-hydroxytetradecenoate ($C_{14:1}$), 3-hydroxytetradecadienoate ($C_{14:2}$), 3-hydroxytetradecatrienoate (C14:3)

unsaturation in these *mcl*-PHA polymers was 3-hydroxytetratrienoic acid ($C_{14:3}$), the mass spectrum of which can be seen in Fig. 2b. Interestingly, the *mcl*-PHA polymers synthesized by Po-14683, Pc-388, and Pp-2442 each maintained $C_{8:0}$ as the major component (55–60%) of their polymers. In fact, their $C_{8:0}$:$C_{10:0}$ ratios were 2.5:1, 2.4:1, and 2.9:1, respectively and resulted in PHA polymers that flowed at room temperature. In contrast, the *mcl*-PHA from Pr-2649 showed the opposite, having $C_{10:0}$ as the major component (48%) and a $C_{8:0}$:$C_{10:0}$ ratio of 0.6:1, which imposed a tacky elastomeric character to this particular polymer. This difference indicates that while Pr-2649 possesses the genetic makeup to synthesize *mcl*-PHA, its PHA synthase genes (*phaC1* and *phaC2*) are sufficiently different to alter its preferred substrate, resulting in *mcl*-PHA polymers with significantly dissimilar monomer content.

Interestingly, those organisms that produced PHB did so less efficiently than those that synthesized *mcl*-PHA. Po-14682 and Po-778 produced 0.1 g/L and 0.7 g/L of polymer, respectively, which translated to productivity values (P; Polymer Yield ÷ CDW × 100) of only 6 and 27%. In contrast, the *mcl*-PHA producers synthesized polymers with P values of between 39% (Pp-2442) and 53% (Pr-2649). Part of this difference must be attributed to the rate at which the fatty acids were metabolized. By recovering the residual fatty acids from each fermentation after bacterial growth, it was found that the PHB producers utilized between 42% (Po-778) and 46% (Po-14682) of the original fatty acids present. On average, this was 32% less than the *mcl*-PHA producers, which used between 61% (Pp-2442) and 69% (Pr-2649) of the starting available carbon source. This discrepancy could be the result of different chemical precursors needed for the formation of PHB and *mcl*-PHA. Normally, PHB is synthesized from the condensation reaction of 2 acetyl-CoA units to produce acetoacetyl-CoA, which is then enzymatically converted to 3-hydroxybutyryl-CoA and polymerized to PHB. The acetyl-CoA is obtained as one product of the β-oxidation reaction of fatty acids. The other product is the fatty acid itself, 2 carbons shorter. It is evident based on the specific polymers produced by each bacterial strain that the PHB producers utilized the acetyl-CoA fraction and the *mcl*-PHA producers, the fatty acid fraction upon β-oxidation. A hint may come from the preferential utilization of the individual fatty acids in the Pollock oil. After growth, the residual fatty acid material was isolated, purified and analyzed by HPLC to determine any trends in fatty acid utilization as they pertain to PHA production. The compositional data from the re-isolated crude Pollock oil after bacterial growth can be seen in Table 1. By comparing the chromatographic peak intensities of each peak to the oleic acid peak, which eluted at 9.4 min, the intensity (peak area) of which was relatively unchanged (±5%) from before to after bacterial growth, it was seen that the PHB producers seemed to preferentially utilize the longer chain fatty acids while the *mcl*-PHA producers utilized the shorter chain fatty acids (Fig. 1). Specifically, the *mcl*-PHA producers caused obvious decreases in the myristic acid (C14:0), palmitic acid (C16:0), and palmitoleic acid (C16:1), while the PHB producers resulted in drops in the EPA and DHA concentrations. The data seem to indicate that the less efficient PHB production system in Po-778 and Po-14682 is the result of diminished acetyl-CoA availability which may be linked to two possibilities: first, it is possible that because acetyl-CoA plays such a central role in basic cellular metabolic processes as a metabolic intermediate and in energy production that at least some of the acetyl-CoA is shuttled away from the PHB biosynthetic mechanism and into other cellular processes, second, a less efficient β-oxidation cycle within the PHB producing strains may also be present resulting in fewer acetyl-CoA molecules being formed for polymer synthesis. Either alone or taken together (more likely), these possibilities would result in a less efficient polymer production system.

A comparison of the molecular weights between the PHB and *mcl*-PHA polymers revealed that, with the exception of the *mcl*-PHA produced by Pr-2649, the number average molecular weights (M_n) of the PHB polymers from both Po-778 and Po-14682 were approximately double the size

Table 4 Molecular weights of PHA polymers synthesized from hydrolyzed crude Pollock oil

Bacterial Strain[a]	PHA Type	M_n (g/mol; ×1000)[b]	M_w (g/mol; ×1000)[b]	M_w/M_n[b]
Po-14682	PHB	195	394	2.02
Po-778	PHB	206	465	2.26
Po-14683	*mcl*-PHA	84	219	2.61
Pp-2442	*mcl*-PHA	86	214	2.49
Pc-388	*mcl*-PHA	100	208	2.08
Pr-2649	*mcl*-PHA	153	343	2.24

[a] For definitions of bacterial strain abbreviations see footnote from Table 1

[b] M_n = number average molecular weight; M_w = weight average molecular weight; M_w/M_n = polydispersity

of the *mcl*-PHA polymers (Table 4). A clue to the cause of these differences is found in the molecular weight distributions of each polymer. The GPC traces of the PHB polymers and the *mcl*-PHA polymers can be seen in Fig. 3. The polydispersity (M_w/M_n) values for both PHB and *mcl*-PHA were 2.02 to 2.61; however, the shapes of the GPC traces were significantly different. The PHB polymers from Po-778 and Po-14682 exhibited Gaussian distributions with elution volume maxima at 10.67 and 10.80 mL, respectively, (Fig. 3a) while all of the *mcl*-PHA polymers exhibited bimodal molecular weight distributions (Fig. 3b). Interestingly, the *mcl*-PHA polymers that were derived from Po-14683, Pc-388 and Pp-2442 all showed the smaller molecular weight class in the bimodal distribution to predominate. In contrast, the *mcl*-PHA from Pr-2649 showed two molecular weight classes that were approximately equal. The higher concentration of the larger molecular weight class caused a shift upward in the overall molecular weight of the polymer which elevated the M_n value for the *mcl*-PHA from Pr-2649.

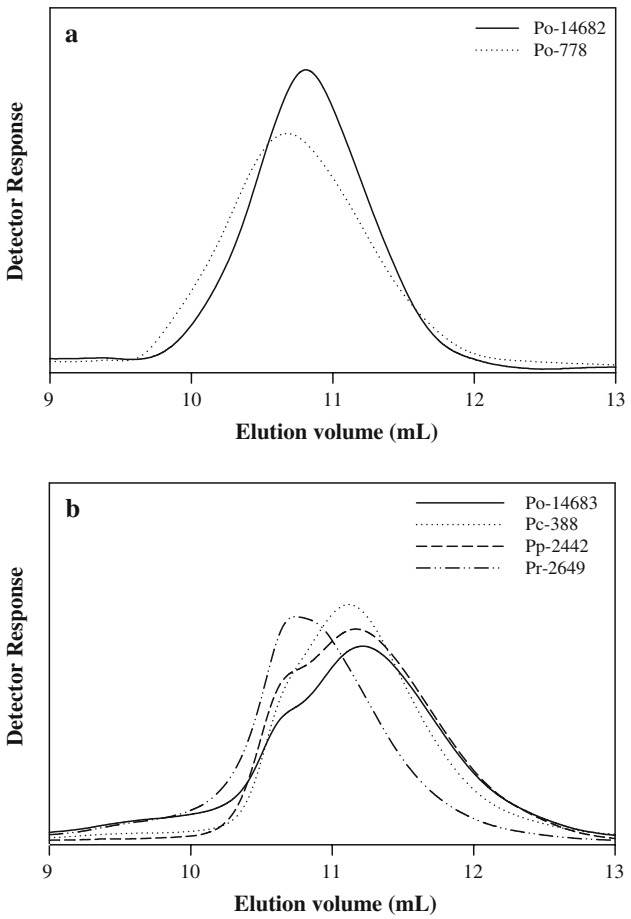


Fig. 3 Gel permeation chromatograms (GPC) of the PHB polymers produced by Po-14682 and Po-778 (**a**) and the *mcl*-PHA polymers produced by Po-14683, Pp-2442, Pc-388 and Pr-2649 (**b**)

Summary

We have demonstrated in this study that crude Pollock oil can be used as a feedstock for the fermentative production of PHA polymers. The repeat-unit compositions of the PHA polymers were determined and showed that polymer properties could be controlled based on the bacterial strain used for synthesis. Two of the bacterial strains studied synthesized PHB, a semi-crystalline thermoplastic with properties comparable to some petrochemical-based plastics. Four strains synthesized *mcl*-PHA, which is entirely amorphous and exhibits elastomeric or free-flowing properties. The ability to produce two types of PHA polymers with widely varying properties under identical growth conditions will allow for the production of controlled *scl*-/*mcl*-PHA polymer blends simply by varying inoculation strategies. In addition, this technology will provide an additional outlet for some fish byproducts, thus conferring additional value to an undervalued, high volume industrial coproduct and at the same time provide an "environmentally benign" polymeric material for industrial use.

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